

What is claimed is:

1. A method for monitoring nucleic acid amplification comprising:
 performing nucleic acid amplification on a target polynucleotide wherein the
 amplification is carried out using any method using a first oligonucleotide probe and a
 second shorter oligonucleotide probe varying in length by at least about 2 base pairs;
 the first probe having a fluorophore;
 the second being complementary with the first probe and having a quencher
 molecule capable of quenching the fluorescence of said fluorophore, the fluorophore
 and quencher being attached on their respective probes at positions which results in the
 quencher molecule quenching the fluorescence of the fluorophore when the probes are
 hybridized,
 wherein the longer probe binds preferentially to the target polynucleotide and
 when preferentially bound to the target polynucleotide the fluorescence intensity of the
 fluorophore is greater than the fluorescence intensity of the fluorophore when
 hybridized to the second probe, and
 monitoring the fluorescence of the fluorophore, the generation of fluorescence
 corresponding to the occurrence of nucleic acid amplification.
2. The method of claim 1 wherein the nucleic acid polymerase is a
 thermostable nucleic acid polymerase.
3. The method of claim 1 wherein the fluorophore on the first probe and
 the quencher molecule on the second probe are on the same hybridized base pair.
4. The method of claim 1 wherein the fluorophore and quencher molecules
 are within about 1 to 3 hybridized base pairs of each other.
5. The method of claim 1 wherein the fluorophore and quencher molecules
 are within 3 or more hybridized base pairs of each other.
6. The method of claim 1 wherein the fluorophore is on the 5' terminal
 nucleotide of the first probe and the quencher is on the 3' terminal nucleotide of the
 second probe.
7. The method of claim 1 wherein the fluorophore is on the 3' terminal
 nucleotide of the first probe and the quencher is on the 5' terminal nucleotide of the
 second probe.
8. The method of claim 1 wherein the second probe is shorter than the first
 probe by deletion of 3 or 3' terminal nucleotides from the nucleotide sequence of the
 first probe.
9. The method of claim 1 wherein the second probe is shorter than the first
 probe by deletion of 3 or more 3' terminal nucleotides from the nucleotide sequence of
 the first probe.

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11. The method of claim 1 wherein the second probe is shorter than the first probe by deletion of 3 or more 5' terminal nucleotides, and deletion of 1 or more 3' terminal nucleotides of the first probe.
12. The method of claim 1 wherein the first and second probes have a
5 disassociation temperature difference of 2 degrees or more.
13. A method for detecting the presence of specific nucleic acid sequences in a prepared nucleic acid sample comprising:
placing a sample of nucleic acids in a suitable solution and incubating with a first oligonucleotide probe and a second shorter oligonucleotide probe varying in length
10 by about at least 2 base pairs;
the first probe having a fluorophore;
the second being complementary with the first probe and having a quencher molecule capable of quenching the fluorescence of said fluorophore, the fluorophore and quencher being attached on their respective probes at positions which results in the
15 quencher molecule quenching the fluorescence of the fluorophore when the probes are hybridized,
wherein the longer probe binds preferentially to the target polynucleotide and when preferentially bound to the target polynucleotide the fluorescence intensity of the fluorophore is greater than the fluorescence intensity of the fluorophore when
20 hybridized to the second probe, and
monitoring the fluorescence of the fluorophore, the generation of fluorescence corresponding to the presence of specific nucleic acid sequences.

adda1

Table I

CV 03 SEQ

	61	71	81	91	1	11	21	31	
N									
3151	SGGDIYHSVS	HARPRWFVFC	LLLLAAGVGI	YLLPNRBASE	CNTACGTRIG	INGCCAGCCC	CCTGATGGGG	GCGACACTCC	ACCATGAATC
N									
3241	ACTCCCCTGT	GAGGAACACTAC	TGCTTCACG	CAGAAAGCGT	CTAGCCATGG	COTTAGTATG	AGTGTCGTGC	AGCCTCCAGG	ACCCCCCCTC
N									
3331	F CCGGGAGAGC	CATAGTGGTC	TGCGGAACCG	GTGAGTACAC	CGGAATTGCC	AGGACGACCG	GGTCCTTTCT	TGGATAAAC	CGCTCAATGC
N									
3421	CTGGAGATTT	GGGCGTGCCC	CCGCAAGACT	GCTAGCCGAG	TAGTGTGGG	TCGCGAAAGG	CCTTGTGGTA	CTGCCTGATA	GGGTGCTTGC
N									
3511	GAGTGCCCCG	GGAGGTCTCG	TAGACCGTGC	ACCATGAGCA	CGAATCCTAA	ACCTCAAGGA	AAACCAAAAC	GTAACACCAA	CCGTGCCCCA

Probes (C1, C2)

← T
 CATAGTGGTC
 TGGCGAACCG

Primer F

Primer R